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Effect of chemicals and plant extract on sclerotial viability and exploration of phytoextracts against *Sclerotium Rolfsii*. Sacc. inciting collar rot of gerbera

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ABSTRACT

The collar rot disease of gerbera caused by *Sclerotium rolfsii* Sacc. is a major challenge for gerbera growers in its successful cultivation under greenhouse conditions. The disease was noticed in the range of 21.60 to 45.20 per cent in different greenhouses from different tahasils of Ratnagiri district (M.S). Among the various chemicals tested for their effect in arresting the germination of sclerotia of *Sclerotium rolfsii* formaldehyde (5%) and ethyl alcohol (40%) showed complete inhibition of sclerotial germination irrespective of soaking period. Similarly, sclerotia exhibited viability for 150 days when stored at ambient temperatures. Among the different plant extracts tested against *S. rolfsii* maximum per cent inhibition of mycelial growth and less number of sclerotia formation was achieved due to soapnut 10 per cent (81.11 % and 12 nos.) which was followed by Neem (56.66% and 18 nos.), Ashwagandha (47.77 and 21nos.)

Key words: - Phytoextracts, formaldehyde, soapnut, sclerotia, sclerotium, neem.

Abbreviations: - PDA – Potato dextrose agar, NOS - number of sclerotia.

1. INTRODUCTION

Gerbera (*Gerbera jamesonii*) is an herbaceous perennial flower crop with long leafless stalks and daisy-like flowers. A native of South Africa, it is a popular cut flower grown throughout the world in a wide range of climatic conditions. Gerbera occupies 500 acres of area in India with production of 50 crore stems and average prices throughout year Rs. 2.5 during 2009-10 (Anonymous, 2010). Maharashtra shares 35 per cent of total gerbera production of India followed by Karnataka (26%), Gujarat and Uttar Pradesh (16% each). In Maharashtra, gerbera is commercially cultivated on large scale mainly in Pune, Nasik, Ahmednagar, crop at Department of Horticulture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli and farmer's greenhouses at Dapoli, Khed and Chiplun tahasils of Ratnagiri district where gerbera was grown on large scale. This disease was found newly emerging problem in commercial cultivation of gerbera creating

a new challenge for gerbera growers of *konkan* region.

2. MATERIAL AND METHODS

2.1. Effect of chemicals and plant extract on viability of sclerotia

Five chemicals and one plant extract were tested with different concentrations for their effect on viability of sclerotia of *Sclerotium rolfsii*. Firstly 30 days old sclerotia collected from cultured Petri plats were soaked in such chemicals and phytoextracts for 30, 60 and 120 minutes. After soaking, sclerotia were washed thrice in distilled sterile water and then transferred in Petri plats containing potato dextrose agar medium. Sclerotia placed on PDA by surface sterilizing in 0.1 per cent HgCl₂ followed by washing thrice in distilled sterile water for each soaking period served as control. The plates were incubated in BOD incubator at 27± 2°C and observed for germination of sclerotia. The experiment was laid out in FCRD with seven treatments and three sub-treatments (three soaking periods). Each treatment was replicated thrice and per cent sclerotia germinated was calculated. Similarly, 30 days old sclerotia were collected and stored for testing their viability at ambient temperature under laboratory condition. At every 15 days interval five sclerotia were transferred on PDA in each Petri plate and observed for their germination. The sclerotial viability at ambient temperature under laboratory condition was studied for 150 days at 15 days interval by following the same technique.

2.2. Efficacy of plant extracts against causal organism *in vitro*

2.2.1. Preparation of phytoextracts

Aqueous phytoextracts were obtained as per the method described by Bhatti (1988). Hundred-gram fresh plant materials were washed thoroughly with sterile distilled water and ground well in 100 ml sterile distilled water. The macerate was then filtered through double layer cotton wool and centrifuged at 4000 rpm, for 5 min. The supernatant was filtered through filter paper. Extracts thus obtained was passed through sintered glass filter to avoid bacterial contamination. This formed the standard plant extracts solution (100 %).

2.2.2. Effect of plant extracts on mycelial growth and sclerotia formation of *S. rolfsii*

The effect of ten different plant extracts on mycelial growth sclerotia formation by *S. rolfsii* was studied by 'Poisoned Food Technique' (Nene and Thapliyal, 1983). All the plant extracts were tested at 10 per cent concentration using potato dextrose agar medium as a basal medium. To obtain 10 per cent plant extract, 90 ml of molten PDA was mixed with 10 ml of standard plant extract in 250 ml conical flask, separately. Twenty milliliter of the medium was then poured in each sterilized Petri plate. Then mycelial discs of 5 mm diameter were cut from seven-day old culture of *Sclerotium rolfsii* with the help of sterilized cork borer and transferred aseptically to the centre of each Petri plate already poured with poisoned medium. Medium devoid of plant extract served as control. Petri plates were incubated at room temperature (27 ± 2° C) for further growth of the fungus. Three replications of each treatment were maintained. The observations for colony diameter and sclerotia formation were recorded until whole of the plate in control treatment was fully covered with mycelial growth. Per cent mycelial growth inhibition was calculated (Horsfall, 1956).

3. RESULTS

3.1. Effect of chemicals and plant extract on sclerotial viability

The data from table 1 revealed that treatment (soaking) of sclerotia of *S. rolfsii* with formaldehyde (5%) and ethyl alcohol (40%) showed complete inhibition of sclerotial germination and were at par with each other. Both formaldehyde (5%) and ethyl alcohol (40%) completely killed the sclerotia irrespective of soaking periods. Both the treatments were statistically significant over rest of the treatments. This was followed by metalaxyl (0.3%) carbendazim (0.1%) captan (0.2%) and soapnut extract (10%) with 8.89, 6.67, 4.44 and 2.22 per cent inhibition of sclerotial germination, respectively.

3.2. Effect of time interval

Effect of time intervals was found to be non-significant for germination of sclerotia.

3.4. Interaction effect

The interactions among time interval (soaking period) and chemicals and plant extract were non-significant for sclerotia germination.

3.5. Effect of time interval on viability of sclerotia at normal environmental conditions

Data presented in table 2 on viability of sclerotia at ambient temperature under laboratory conditions showed that the sclerotia of *S. rolfsii* were viable up to 150 days (till the end of the present investigation).

Table 1

Effect of time interval, chemicals and plant extract on viability of sclerotia of *S. rolfsii*

Treatments	Name of chemicals with concentration	Per cent Sclerotial germination inhibited			Mean
		S ₁	S ₂	S ₃	
T ₁	Formaldehyde (5%)	100.0 (90.00)*	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T ₂	Carbendazim (0.1%)	0.00 (0.00)	6.67 (8.86)	13.33 (17.71)	6.67 (8.86)
T ₃	Captan (0.2%)	0.00 (0.00)	6.67 (8.86)	6.67 (8.86)	4.44 (5.90)
T ₄	Metalxyl (0.3%)	6.67 (8.86)	13.33 (17.71)	6.67 (8.86)	8.89 (11.81)
T ₅	Ethyl alcohol (40%)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T ₆	Soapnut (10%)	0.00 (0.00)	0.00 (0.00)	6.67 (8.86)	2.22 (2.95)
T ₇	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean		29.52 (26.98)	32.38 (30.77)	33.33 (32.04)	31.75 (29.93)
			S.E.m ±	C.D. at 1%	
S			2.00	7.62	
T		Sig.	3.05	11.64	
S X T			5.28	20.15	

(*Figures in parenthesis are arcsin values.)

Where, S₁- 30 min., S₂- 60 min., S₃- 120 min.

Table 2

Effect of time interval on viability of sclerotia at ambient temperature under laboratory condition

Sr. No.	Days interval	Number of sclerotia inoculated/ replication	Number of sclerotia germinated					Mean
			R I	R II	R III	R IV	R V	
1.	30	5	5	5	5	5	5	5
2.	45	5	5	5	5	5	5	5
3.	60	5	5	5	5	5	5	5
4.	75	5	5	5	5	5	5	5
5.	90	5	5	5	5	5	5	5
6.	105	5	5	5	5	5	5	5
7.	120	5	5	5	5	5	5	5
8.	135	5	5	5	5	5	5	5
9.	150	5	5	5	5	5	5	5

Table 3*In vitro* efficacy of Phytoextracts against mycelial growth and sclerotia formation of *Sclerotium rolfsii*.

Tr.No.	Treatment	Conc (%)	Plant parts used	Mean colony diameter* (cm)	Per cent inhibition over control	No. of sclerotia formed
T ₁	Sarpagandha	10	Leaves	6.2	31.11	29
T ₂	Glyricidia	10	Leaves	6.4	28.88	33
T ₃	Tulsi	10	Leaves	5.3	38.88	25
T ₄	Neem	10	Leaves	3.9	56.66	18
T ₅	Onion	10	Bulb	6.0	33.33	30
T ₆	Nilgiri	10	Leaves	5.9	34.44	28
T ₇	Castor	10	Leaves	6.0	33.33	31
T ₈	Soapnut	10	Fruit	1.7	81.11	12
T ₉	Heena	10	Leaves	5.3	41.11	27
T ₁₀	Ashwagandha	10	Leaves	4.7	47.77	21
T ₁₁	Control			9.0	-	65
S. Em \pmC.D at 1%				0.16 0.65		

*Mean three replications

3.6. *In vitro* efficacy of plant extracts against *Sclerotium rolfsii* Sacc.

Ten different plant extracts were tested against the collar rot causing fungus *S. rolfsii* on gerbera *in vitro*. The data on inhibitory effect of plant extracts on mycelial growth and sclerotia formation of the *S. rolfsii*. is presented in Table 3. It is revealed that all plant extracts at 10 per cent concentration were found significantly effective in inhibiting mycelial growth and sclerotia formation of *S. rolfsii*. Among all the treatments, maximum per cent inhibition of *Sclerotium rolfsii* over control and less number of sclerotia formation was achieved due to Soapnut (81.11% and 12 nos.) and was followed by Neem (56.66% and 18 nos.), Ashwagandha (47.77% and 21nos.) and Heena (41.11% and 27 nos). Tulsi, Nilgiri, Castor, Onion, Sarpagandha and *Glyricidia* recorded poor inhibition of colony diameter to the tune of 38.88%, 34.44%, 33.33%, 33.33%, 31.11% and 28.88% with 25, 28, 31, 30, 29 and 33 nos. of sclerotia formed, respectively over control.

4. DISCUSSION**4.1. Effect of chemicals and plant extract on sclerotial viability**

Formaldehyde (5%) and ethyl alcohol (40%) showed complete inhibition of sclerotial germination and were at par with each other. Both chemicals were completely killed the sclerotia irrespective of soaking periods. This was followed by metalaxyl (0.3%) carbendazim (0.1%) captan (0.2%) and soapnut extract (10%) with 8.89, 6.67, 4.44 and 2.22 per cent inhibition of sclerotial germination, respectively. The present results are comparative to those reported by Okonkwo (1988) who reported effectiveness of formaldehyde at various concentrations against stem-rot fungus, *Sclerotium rolfsii*. Similarly, Mahmood *et al.* (1977) observed that formaldehyde completely inhibited the fungal growth of *Sclerotium rolfsii* and formaldehyde was most effective in killing sclerotia of the test fungus.

4.2. Effect of time interval on viability of sclerotia at normal environmental conditions

The sclerotia of *S. rolfsii* were viable up to 150 days. The present results are in close conformity to those reported by Abeygunawardena *et al.* (1957) who studied factors affecting germination of sclerotia and mycelial growth of *Sclerotium rolfsii* Sacc.

4.3. *In vitro* efficacy of plant extracts against *Sclerotium rolfsii* Sacc.

Maximum per cent inhibition of *Sclerotium rolfsii* over control and less number of sclerotia formation was achieved due to Soapnut (81.11 % and 12 nos.) and was followed by Neem (56.66% and 18 nos.), Ashwagandha (47.77% and 21nos.) and Heena (41.11% and 27 nos). Tulsi, Nilgiri, Castor, Onion, Sarpagandha and *Glyricidia* recorded poor inhibition of colony diameter to the tune of 38.88, 34.44, 33.33, 33.33, 31.11 and 28.88 per cent with 25, 28, 31, 30, 29 and 33 nos. of sclerotia formed, respectively. These results are in line with the findings of Haralpatil and Raut (2008) who reported that the Neem plant extract was effective for inhibition of mycelia growth and sclerotia formation of *Sclerotium rolfsii* Sacc.

5. CONCLUSION

From the results it is concluded that formaldehyde (5%) and ethyl alcohol (40%) are the most effective chemicals which can completely inhibit the germination of sclerotia. Similarly, metalaxyl (0.3%) carbendazim (0.1%) captan (0.2%) and soapnut extract (10%) can also inhibit the sclerotial germination to a certain extent. Sclerotia of the pathogenic fungus *S. rolfsii* may survive for at least 150 days at ambient temperature under laboratory conditions. Fruit extract of soapnut, neem and ashwagandha are emerged as promising inhibitors of causal fungus of gerbera collar rot.

Disclosure statement

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Informed consent

Not applicable.

Ethical approval

The ethical guidelines for plants & plant materials are followed in the study.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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Data and materials availability

All data associated with this study are present in the paper.

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